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COLLEGE OF MEDICINE
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The blood microbiota in health and disease

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The blood

- Circulation – 5L
- One microliter blood contains:
 - 4-6 million erythrocytes
 - 200-500,000 thrombocytes
 - 4-11,000 leukocytes

I. Methodological challenges, considerations

Challenges when working with blood

- Quick processing time
- Skin contamination
- Catheter contamination
- Sample amount
- Storage
- Anticoagulants

Detection of microbes in blood

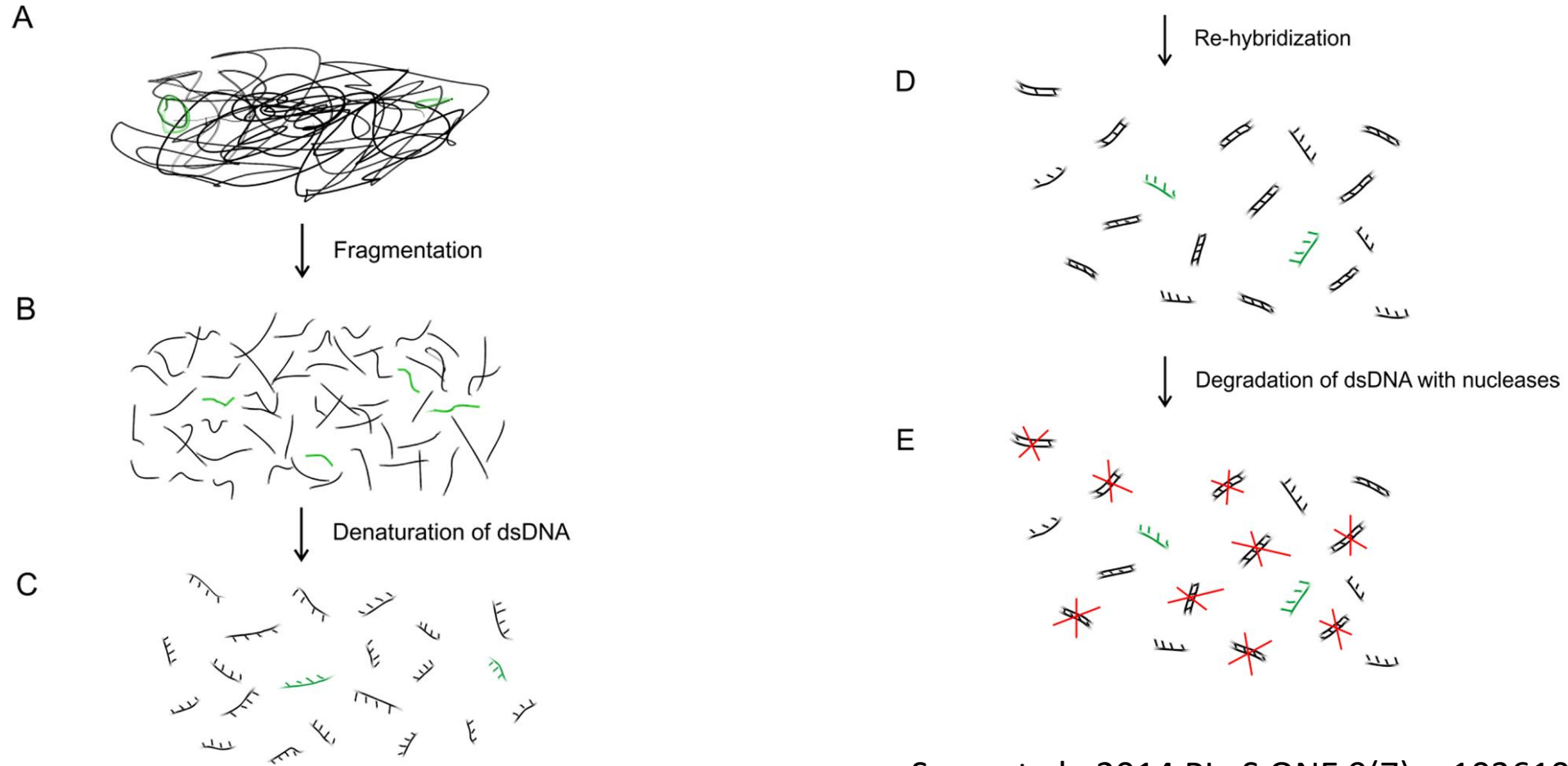
- Viability
- Culturable/cultured pathogens only
- Typically monomicrobial detection
- Does not detect viruses
- 50-90% the infectious agent remain unknown
- Takes several days
- Requires large volumes of blood (20-40 ml; molecular/metagenomics studies require 0.5-2 ml)



Methodological considerations for metagenomics on blood samples

- Can detect broad variety of microbes
- Large amount of human DNA
- 16S sequencing
- Shotgun sequencing

Methodological considerations – human background DNA



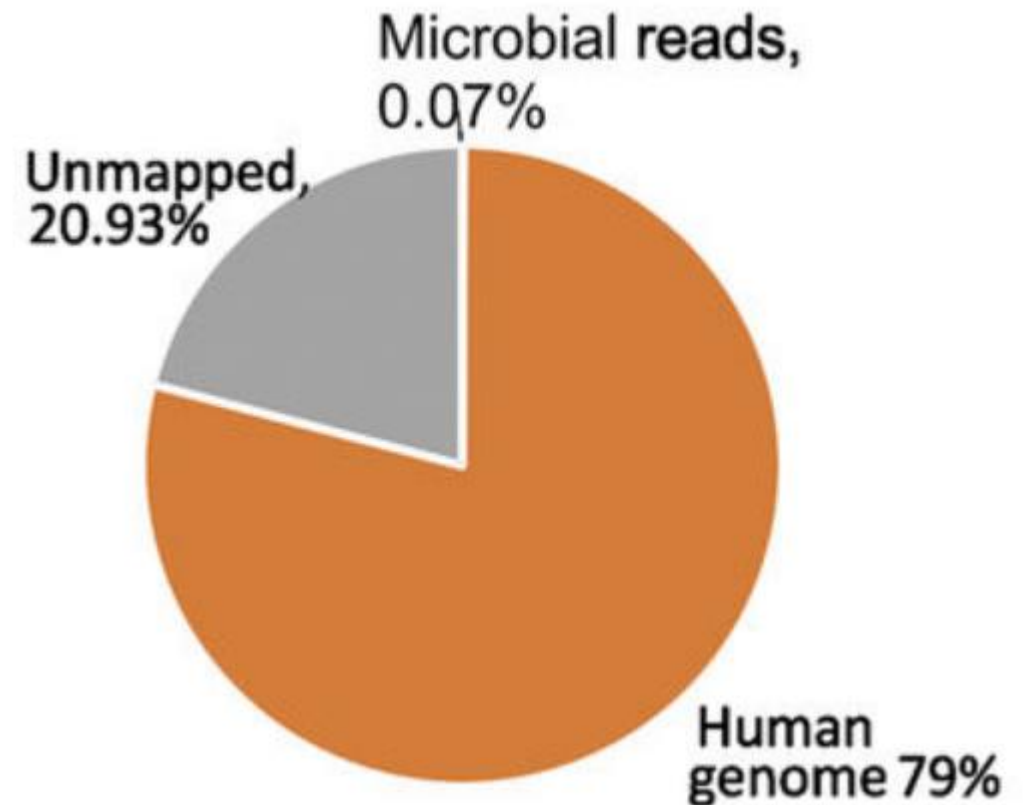
Methodological considerations – 16S sequencing

- High throughput
- Fast
- Culture-independent
- Can identify all bacteria

- Species level identification not reliable
- Does not detect fungi/viruses
- Primer biases

Methodological considerations – shotgun sequencing

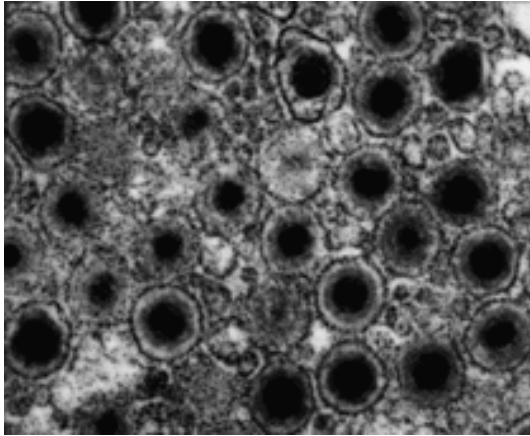
- Expensive
- Mostly sequencing human genome
- Larger starting material
- Huge amount of data
- Bacteria, fungi, viruses
- Resistance genes



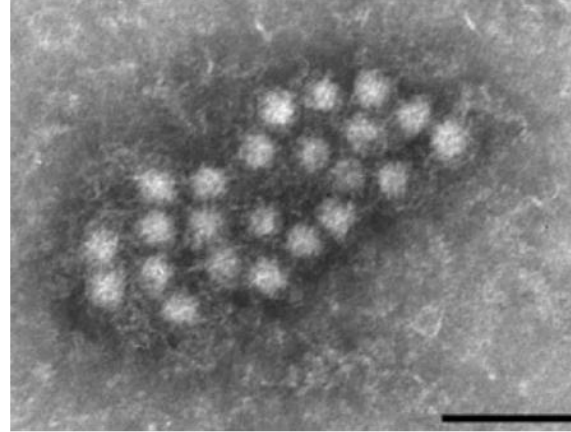
II. Blood microbiota in healthy population

Blood was thought to be sterile

- Viruses / viral segments / phages are often present in healthy people
- High prevalence in the population
- Most often no symptoms present

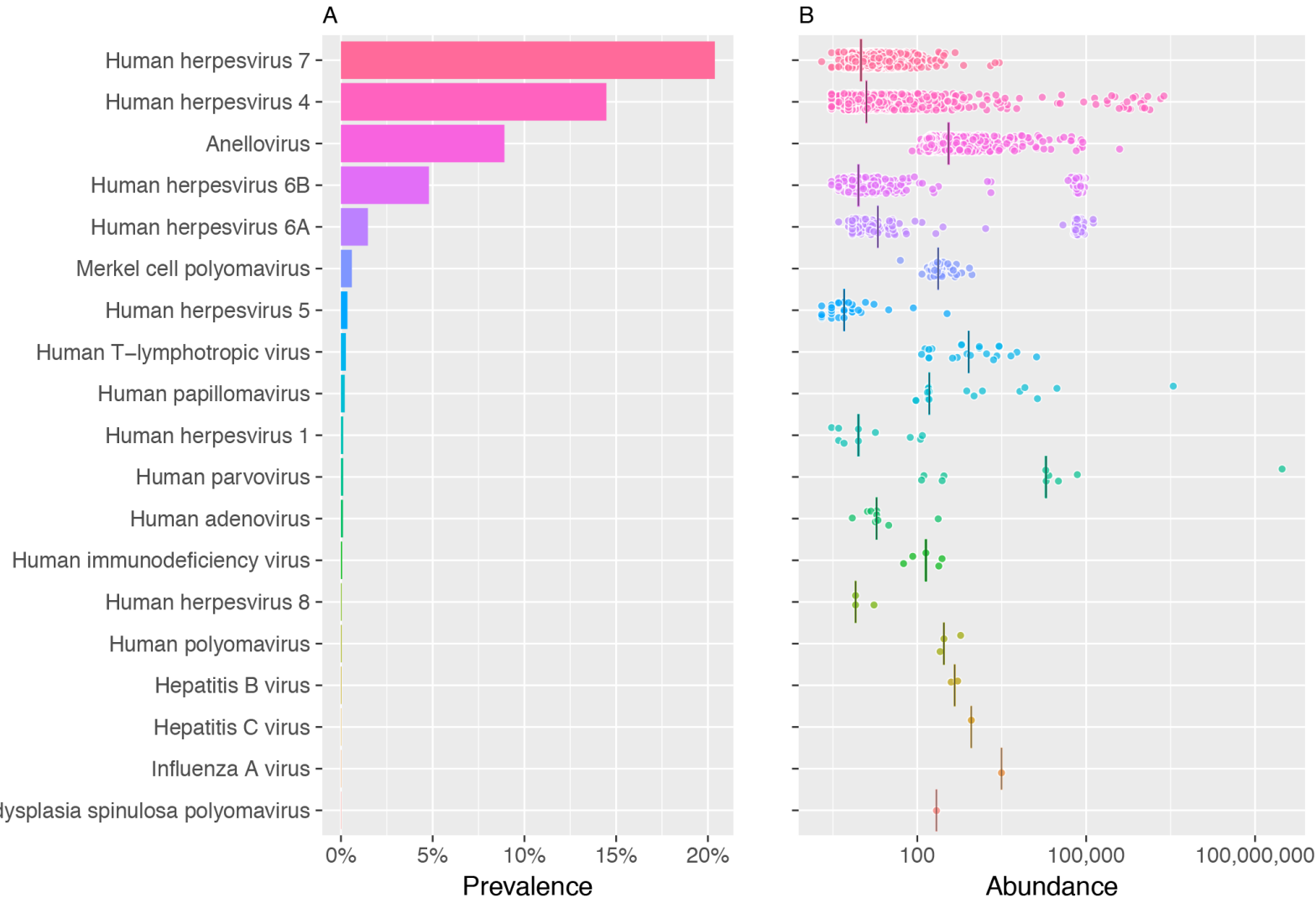


HHV7 (Guerrero&Bacon)



TTV (Itoh et al., 2000)

The blood virome



- 8000 participants
- Cell free DNA
- Shotgun sequencing
- ~40% presumed healthy
- ~60% with common disorders (respiratory, cardiovascular, metabolic disorders)

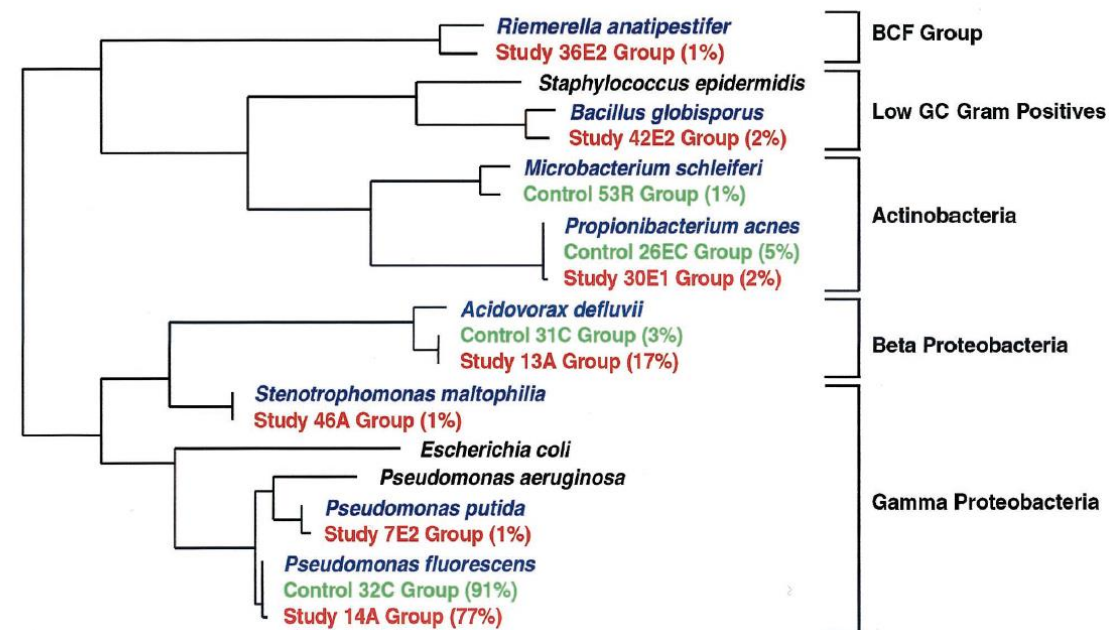
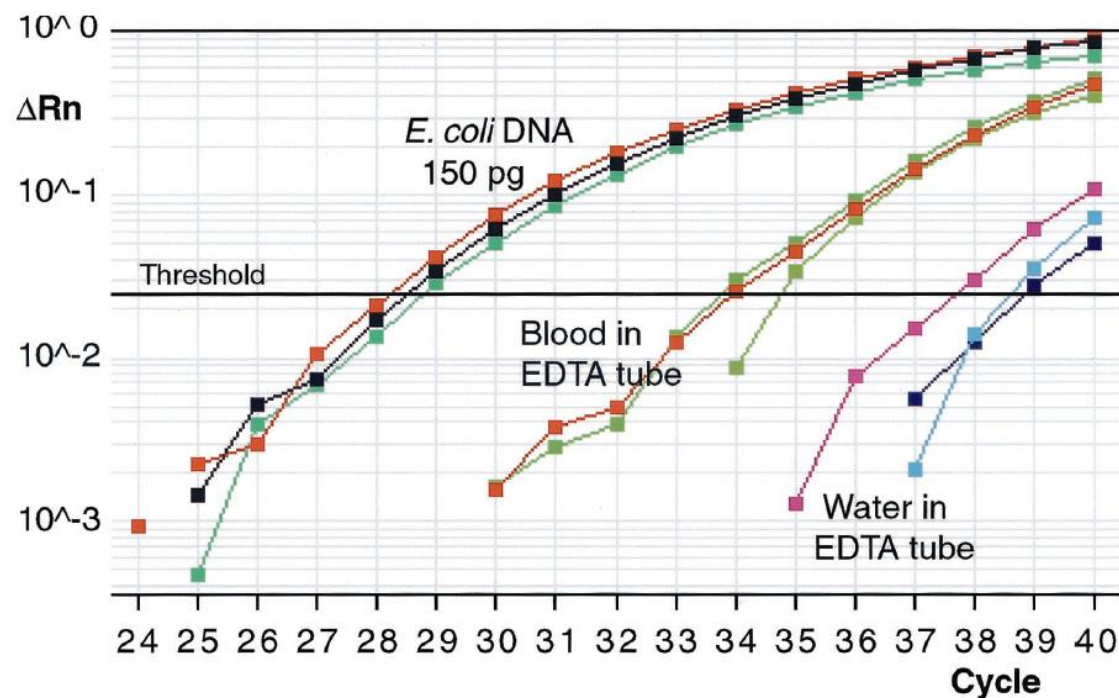
Bacteria in healthy blood?

JOURNAL OF CLINICAL MICROBIOLOGY, May 2001, p. 1956–1959
0095-1137/01/\$04.00+0 DOI: 10.1128/JCM.39.5.1956–1959.2001
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Vol. 39, No. 5

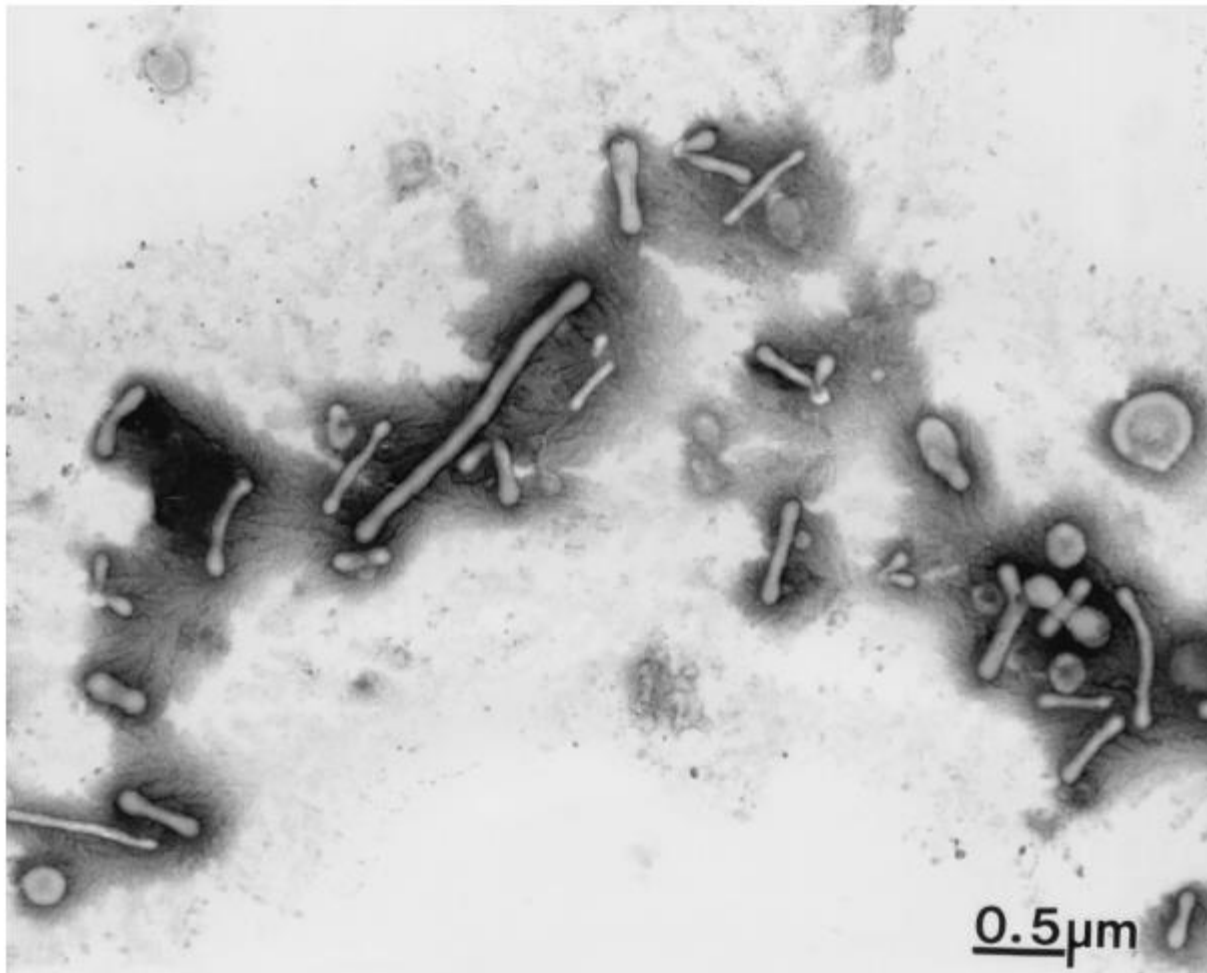
Does Blood of Healthy Subjects Contain Bacterial Ribosomal DNA?

SIMO NIKKARI,^{1,2*} IAN J. McLAUGHLIN,³ WANLI BI,³ DEBORAH E. DODGE,^{3†} AND DAVID A. RELMAN^{1,2}



Are There Naturally Occurring Pleomorphic Bacteria in the Blood of Healthy Humans?

Richard W. McLaughlin,¹ Hojatollah Vali,¹ Peter C. K. Lau,² Roger G. E. Palfree,¹ Angela De Ciccio,¹ Marc Sirois,³ Darakhshan Ahmad,⁴ Richard Villemur,⁵ Marcel Desrosiers,⁵ and Eddie C. S. Chan^{1*}



Stenotrophomonas maltophilia

- Electron microscopy
- 16S PCR
- FISH

Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing

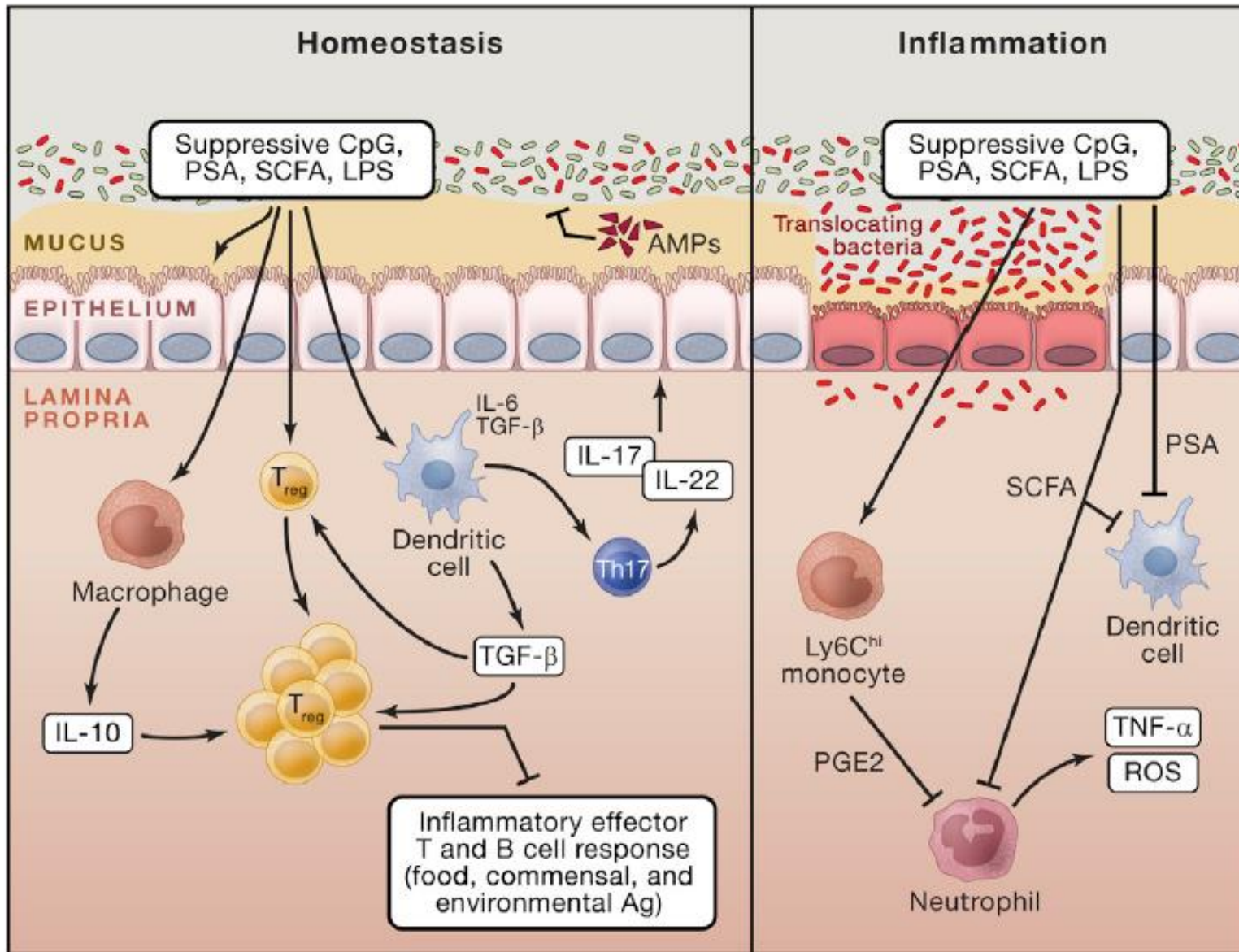
Sandrine Païssé,^{1} Carine Valle,^{1*} Florence Servant,¹ Michael Courtney,¹ Rémy Burcelin,^{1,2}
Jacques Amar,^{1,3*} and Benjamin Lelouvier^{1*}*

Metagenomics analysis of red blood cell and fresh-frozen plasma units

*Pierre Lau,^{1,2} Samuel Cordey,³ Francisco Brito,⁴ Diderik Tirefort,¹ Thomas J. Petty,⁴
Lara Turin,³ Arthur Guichebaron,^{1,2} Mylène Docquier,⁵ Evgeny M. Zdobnov,⁴
Sophie Waldvogel-Abramowski,¹ Thomas Lecompte,¹
Laurent Kaiser,³ and Olivier Preynat-Seauve^{1,2}*

III. Blood microbiota in disease

The “intestinal firewall”



Belkaid Y, Hand TW.

Cell. 2014 157(1):121-41.

Bacterial translocation can occur in several diseases

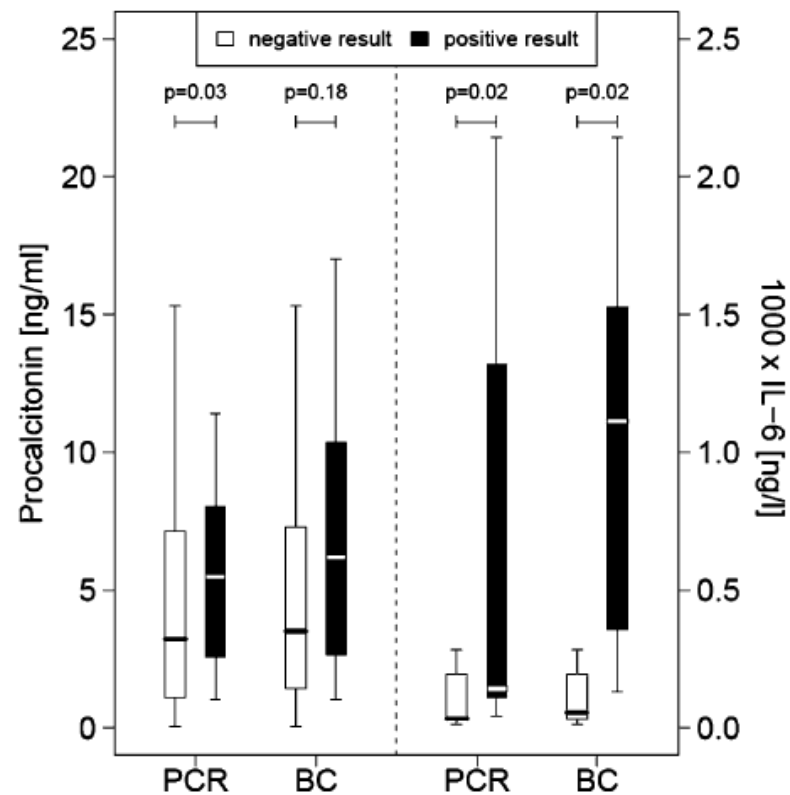
Table 2. Studies on BT in humans.

Clinical condition	Method for testing BT
Burn injury	BC, L/M ratio
Trauma/haemorrhagic shock	BC, MLN, endotoxin, L/M ratio
Endotoxin	L/M ratio
Obstructive jaundice	MLN
Acute pancreatitis	MLN, BC, L/R ratio
Bowel transplant	BC, stool, liver culture
Liver cirrhosis	MLN
Intestinal obstruction	MLN, serosa
Crohn's disease	MLN, serosa
Organ donors	MLN, BC, endotoxin
Elective surgery	MLN, serosa
Aortic aneurysm repair	MLN, serosa
Cardiopulmonary bypass	Endotoxin, L/M ratio
Heart failure	Serum endotoxin
Colchicine overdose	BC
Neutropenia	BC
Malignancy	BC

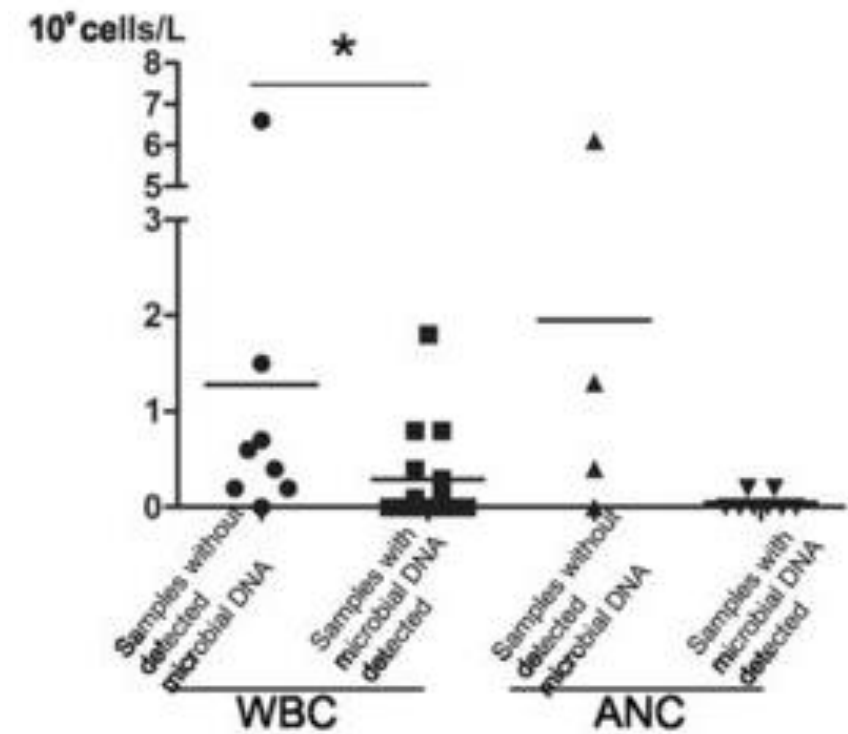
- Hospitalization time, mortality ↑
- Clinically relevant?
- Prognostic impact?
- Methodology?
- Culture/culture independent?
- Patient/control population?

Marc J. Struelens

Detection of microbial DNAemia: does it matter for sepsis management?



Bloos et al., Intensive Care Med (2010) 36:241–247



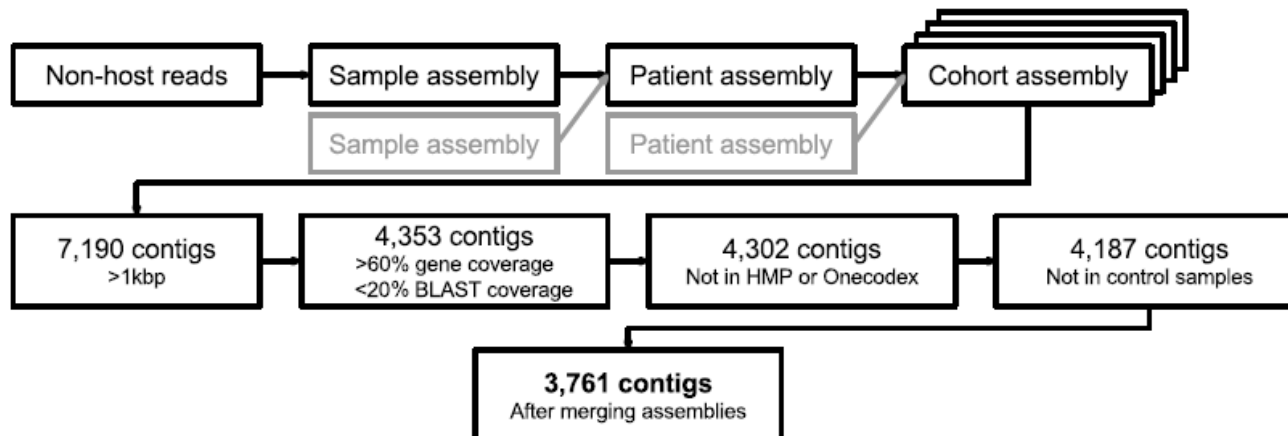
Gyarmati et al., Sci Rep (2016) 6:23532

Microbes in blood with different underlying conditions

Numerous uncharacterized and highly divergent microbes which colonize humans are revealed by circulating cell-free DNA

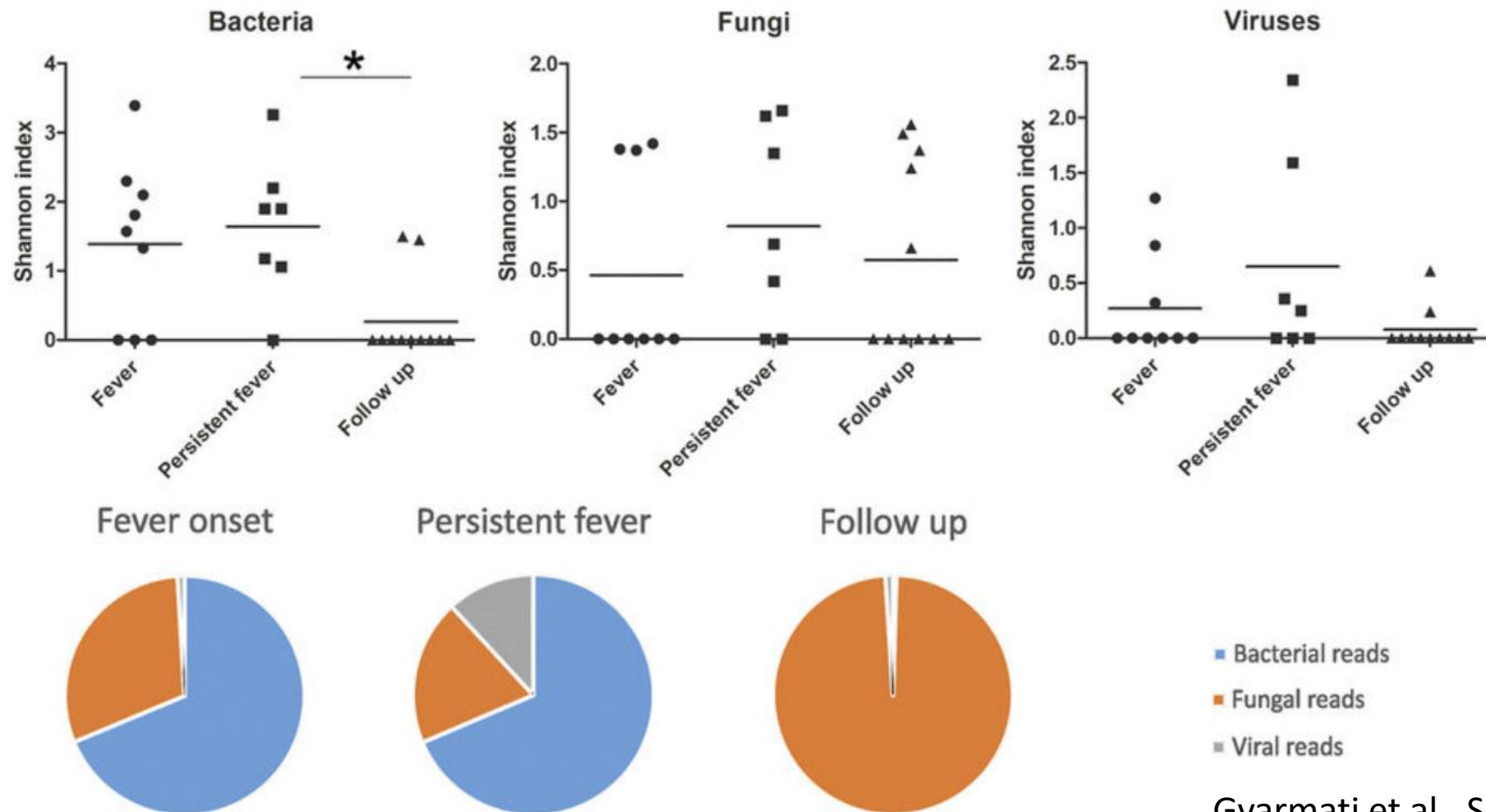
Mark Kowarsky^a, Joan Camunas-Soler^b, Michael Kertesz^{b,1}, Iwijn De Vlaminc^b, Winston Koh^b, Wenying Pan^b, Lance Martin^b, Norma F. Neff^{b,c}, Jennifer Okamoto^{b,c}, Ronald J. Wong^d, Sandhya Kharbanda^e, Yasser El-Sayed^f, Yair Blumenfeld^f, David K. Stevenson^d, Gary M. Shaw^d, Nathan D. Wolfe^{g,h}, and Stephen R. Quake^{b,c,i,2}

www.pnas.org/cgi/doi/10.1073/pnas.1707009114



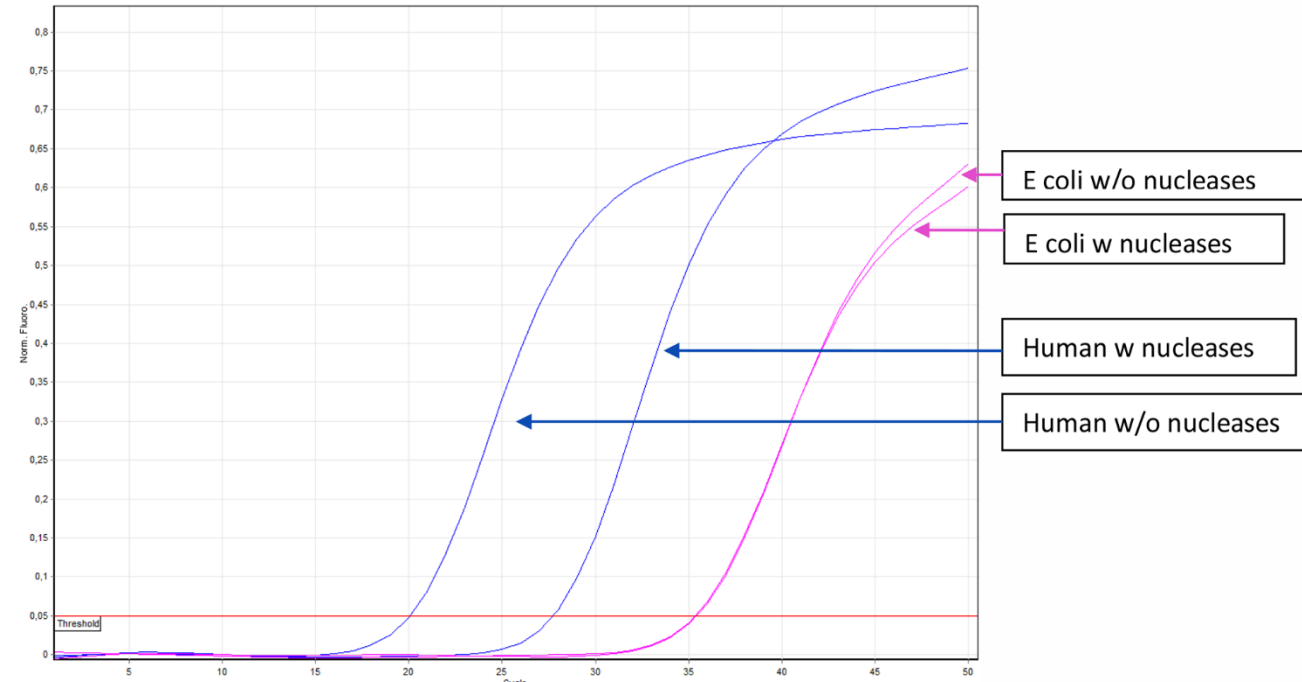
- Heart transplant
- Lung transplant
- Bone marrow transplant
- Pregnancy
- High diversity of phages
- Torque Teno Virus

Blood microbiota in leukemia



Possible solutions

- Remove/decrease human background DNA
- Sequence longer fragments
- Validation of sequencing method
- Filter vs human genome
- Careful selections of controls
 - 16S: measure relative abundances (control vs sample)
 - Shotgun: Phylogeny (control vs sample)
- Optimize 16S PCR



Song et al., 2014 PLoS ONE 9(7): e103610

Summary

- Caution when interpreting data
 - Careful selection of controls
 - Consideration of appropriate extraction/sequencing methods
 - Limited knowledge and evidence of clinical significance
-
- Pathogen-specific treatment
 - Invaluable in the treatment of critically ill / blood culture negative patients
 - Patients with unknown etiology

at a stage of pregnancy when the major risk of abortion had passed. The food supplements were given in the form of tablets and capsules, the only method by which such a large nutritional experiment was possible, and no doubt it was true that natural foods would be even more effective, so that the results, striking as they were, represented rather the minimal than the maximal influence of good nutrition. He added some up-to-date figures relating to changes in infant birth weights, prematurity rates, and toxæmia rates since the war began. The study had been carried out by Dr. Hargreaves at the British Postgraduate Medical School on "booked" cases during 1937 (1,597 cases), 1938 (1,765), 1940 (954), 1941 (846), and 1942 (662). The average birth weights of full-time children had shown a decline since the pre-war years from 7.3 to 7.1 lb. in primigravidae and from 7.6 to 7.4 lb. in multigravidae. The percentage prematurity rates had declined, though not so consistently, from 19.7 to 14.2 in primigravidae and from 16.7 to 13.6 in multigravidae. The percentage toxæmia rates had shown no consistent change.

Prof. BARD, in his reply, said that the appointment of paediatricians to emergency hospitals was desirable, but this would be only a wartime measure, and what he desired to see was appointments of the type recently made in Newcastle, where a doctor had been appointed "Professor of Child Health." He had been interested in Prof. Young's figures. The incidence of toxæmia in primigravidae in Aberdeen in booked cases was 8%, which was very close to the London figure, and he had always understood that there was relatively little toxæmia in maternity cases in London. In a small series of cases in private practice the toxæmia rate was only 3%, or less than half the hospital figure. The percentage prematurity rate in Aberdeen among hospital cases was 23, but in private practice only 8.

Correspondence

What is "Sterile Blood"?

SIR.—Recent contributors to the *Journal* have raised the above question and answered it to their seeming satisfaction, but not without leaving an opening for legitimate criticism. The question as to what constitutes "sterility" is evidently a matter of some perplexity. This is borne out by the report of four experts from the Blood Transfusion Service of the Medical Research Council as published in the *Journal* of March 21, 1942. These workers eventually decided that the ordinary bacteriological tests for the sterility of plasma were of little value, and they discarded them in favour of the naked eye as a check on their plasma storage technique.

Now the experienced bacteriologist may be able to say that cloudiness of plasma or serum means contamination and that clearness argues for probable sterility, but it is a departure from our usual scientific standards to dispense with proof of absolute sterility. It is quite obvious that in dealing with blood the bacteriologist is missing the free use of his autoclave, and that when he speaks of sterile blood he does not mean the same thing as when he refers to a sterile dressing. Everyone knows what is meant by the usual sterile fluids; they have been boiled or otherwise sterilized in order to kill off all forms of life within them. On the other hand, "sterile blood," although free from bacteria, is still a living tissue, but the activities of the living elements in stored blood have so far not had much consideration. There is in fact a widespread belief that the changes which take place in clean stored blood are purely physico-chemical, when as a matter of fact they must include the biological reactions of living things. It becomes necessary therefore to differentiate between those changes which are due to the inherent vital elements in blood and those which are due to contamination. Nor should we forget that the value of blood for many transfusion purposes is greatly diminished by the present storage processes, which aim at suppressing the extraneous forms of life but at the same time destroy the inherent vitality of the blood cells.

This vital aspect of blood was recognized by Croxibie and Scarborough,¹ who, among other criteria of the viability of

the white blood corpuscles, described a curious "spinning" of the granules of the cytoplasm. This rapid oscillatory movement stops immediately when the cell is overheated—in short, when living protoplasm enters into its dead state. I have confirmed this observation, but I have gone further and found that, as the white cells break up, these spinning and, as I hold, living bodies can be seen floating free in the blood serum. With suitable technique, such as the use of dark-ground illumination under a high-power objective, it is also possible to demonstrate that there are other highly motile bodies in disintegrating "sterile blood."

We are faced then with the need for a more intensive investigation of the biological phenomena of stored blood which has been pronounced "sterile." It is clear also that the word "sterile" is not a suitable term in this connexion, because the state of sterility is inconsistent with the presence of vital elements in stored blood.—I am, etc.,

Medical School, University of Capetown. M. R. DRENNAN.

Rehabilitation Centres

SIR.—I enclose a brief memorandum which expresses the general opinion of the members of the British Orthopaedic Association in regard to rehabilitation, and I am doing so at the request of some of the senior members of the Association. We feel that it should be clearly understood that the orthopaedic surgeons of the country are wholly in favour of this constructive medical and sociological movement.

I think we all feel that rehabilitation should begin very soon after the accident or wound, and be carried on throughout the patient's period in hospital in the form of occupational therapy and gymnastic or remedial exercises. There remains as a logical and very necessary development the organization of Rehabilitation Centres where the final and more concentrated stages of rehabilitation are given to enable the patient to be returned to the Services or to industry with the least possible delay.—I am, etc.,

G. R. GIRDLESTONE,
Headington, Oxford. President of the British Orthopaedic Association.

*. The memorandum runs as follows:

The members of the British Orthopaedic Association recognize the value of measures already adopted by the Ministry of Health in introducing rehabilitation into E.M.S. orthopaedic, fracture, and accident services. They are not, however, satisfied that the later stages of rehabilitation have been adequately developed throughout the country; and for this particular purpose they urge the importance of establishing special Rehabilitation Centres, in association with existing orthopaedic, fracture, and accident services; under the same administrative and surgical control, but as a rule geographically separated in order to avoid the hospital atmosphere; and affording all facilities needed for the completion of treatment right up to the stage when work can be resumed. From undoubted evidence of grave wastage of man-power through accidents, they believe that the developments recommended are of immediate urgency in the prosecution of the war.

Listerism

SIR.—I have read the correspondence on the Lister technique in your issues of Sept. 5 and 26 and Oct. 3. A good case made out can't have too much backing, but there seems some confusion as to Listerism among others who think aseptic surgery is different from what Lister taught. The three main principles of Listerism are: (1) disinfection of the skin, (2) destruction or inhibition of the germs in the wound, and (3) prevention of the entrance of germs into the wound. Lister used antiseptics to produce asepsis, and he introduced dressings and a "protective" (oiled silk). We are indebted to him for the undermentioned: In 1866 he used crude creosote for wounds and banished pyæmia from his wards. He made a "plaster-of-Paris putty" for a compound fracture and covered it with tin foil, and so paved the way to the present-day method. (This was at a time when there was no bacteriology.) He tied the carotid artery of a horse under antiseptic conditions with good results, using first kangaroo tendon; this work then led him to chromic acid catgut. He invented a spray, and this led to aerial disinfection. He taught the immobilization of wounds and fractures. He showed the possibilities of skin grafting. He insisted on sharp knives, so as not to bruise tissues. He showed the correct way of giving chloroform without a death. He introduced drainage of wounds. He was the

We are faced then with the need for a more intensive investigation of the biological phenomena of stored blood which has been pronounced "sterile." It is clear also that the word "sterile" is not a suitable term in this connexion, because the state of sterility is inconsistent with the presence of vital elements in stored blood.—I am, etc.,

Medical School, University of Capetown.

M. R. DRENNAN.

Acknowledgements

- Christian Kjellander (Karolinska Hospital)
- Lars Öhrmalm (KH)
- Carl Aust (KH)
- Mats Kalin (KH)
- Christian Giske (Karolinska Institute)
- Yajing Song (UIC, Royal Institute of Technology)

